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SURVIVAL OF MICRO-ORGANISMS IN SPACE

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THIS article describes the first successful direct exposure of unprotected terrestrial micro-organisms to the environment of space, and their recovery. In the course of genetic and other studies, biological materials have previously been flown on board balloons[1,2], rockets[3] and satellites[1,3–5], but the organisms were sealed in protective vessels within the vehicle, and were therefore not directly exposed to the space environment.

Up to the present time the survivability of micro-organisms in space has been a matter for speculation and extrapolation of terrestrial laboratory data[6–14]. The space environment has been viewed mainly as one extremely hostile to all forms of life, in part due to the high flux of biologically lethal ultra-violet and ionizing radiation. However, some indirect evidence concerning the existence of extraterrestrial life has been found by physiochemical methods[15]. The degree to which such factors as extreme desiccation and very low temperatures may offset the lethal agents has not been completely assessed, though much work has been done[6,9,12,14]; the general view has been that these will not cause the rapid death of known life forms. The degree to which agents such as shielding or special chemicals may offer protection is totally unexplored.

The subject is not merely an academic one, containing as it does fundamental questions concerning the spread and origin of life throughout the universe, and there is immediate relevance to the present space exploration programme in respect to the extremely difficult and costly efforts to avoid the contamination of other planets with terrestrial microflora. In this area definitive knowledge is badly needed concerning the survivability of surface contaminants surviving sterilization of space ships or picked up during launch, and on the improbable but theoretically possible event of contamination occurring in space.

With these questions in mind, a programme was initiated for investigation of the presence and survivability of micro-organisms in space, as a subsidiary project to

the Dudley Observatory micrometeorite research programme.

Two flight experiments will be described, one using a rocket for short-term exposure at high altitude, the other a balloon for longer exposure at lower altitude. Additional laboratory experiments were performed to throw more light on the cause of the inactivation found.

Rocket-borne Exposure Experiment

The rocket experiment utilized an *Aerobee* rocket flown at the White Sands proving ground and took place on November 16, 1964. The payload was a micrometeorite collection device developed by the Ames Laboratory of the National Aeronautics and Space Administration.

Sample preparation. The samples consisted of sterile surfaces of autoclaved nylon-reinforced 'Millipore' filter disks for the collection of micro-organisms during flight, and also additional surfaces coated with dried preparations of various terrestrial micro-organisms; these were: *Bacillus subtilis* spores, *Chlorella pyrenoidosa* cells, poliovirus type III (wild), and *Escherichia coli* bacteriophage T_1. Unfortunately the sterile surfaces and the *B. subtilis* and *Chlorella* specimens were lost during the flight; details of their preparations are therefore omitted.

Nylon-reinforced 'Millipore' filter membranes (450 mμ porosity), each approximately 1 cm² in area, were cemented to lucite plates and autoclaved. Aqueous suspensions (consisting of the appropriate culture media) of the viruses were prepared and 0·1 ml. volumes were spread on to these surfaces. These were vacuum dried at room temperature 11 days before launch. The samples were prepared in two identical parts, one of which was shielded with heat-sterilized aluminium foil 38μ thick. Duplicate sets were prepared. Both were stored under vacuum in their collection boxes. Each box had a bellows switch which permitted detection of any serious vacuum leaks. The ground set was for laboratory control of the loss of viability or infectivity due to the drying procedure and storage at room temperature on the ground throughout the experimental period; the flight set was flown on the rocket.

Rocket flight exposure. The rocket flight exposure and collector experiments were carried out in one of four compartments within each of two identical collection boxes. Each box had an exposure area of approximately 930 cm². The payload was similar in design to the *Venus* 'Fly Trap' micrometeorite collector[16]. Twelve evacuated collection boxes were mounted on three hydraulically actuated arms. They were protected by a cylindrical aluminium shroud during launch and upward travel through the atmosphere. At altitude the shroud was hydraulically elevated and the collection boxes were extended on their arms until their exposure areas were

approximately perpendicular to the spin axis of the rocket.

The *Aerobee* rocket was launched at about 11.20 a.m. Mountain Standard Time on November 16, 1964, from the White Sands Missile Range at White Sands, New Mexico. An approximate altitude-time profile for this flight is shown in Fig. 1. The exposure began at an altitude of approximately 80 km and the rocket reached a height of 155 km at apogee. The collector closed when the payload had fallen to about 106 km. The samples were exposed nearly directly upward for approximately 233 sec. Coning of the rocket was negligible. Unfortunately the collector malfunctioned and the collection boxes were not resealed. It also appears to have reopened violently a few seconds before the parachute deployed at an altitude of about 4.25 km. When the parachute deployed, the arms were slammed shut again. The collector was recovered 4 days after the flight. The box containing the biological samples appeared to have suffered the least damage although it was separated from its cover by about 1 cm and the micrometeorite collection surfaces were dirty. The thin nitrocellulose collection films flown in the same box as the biological experiments were partially intact, suggesting

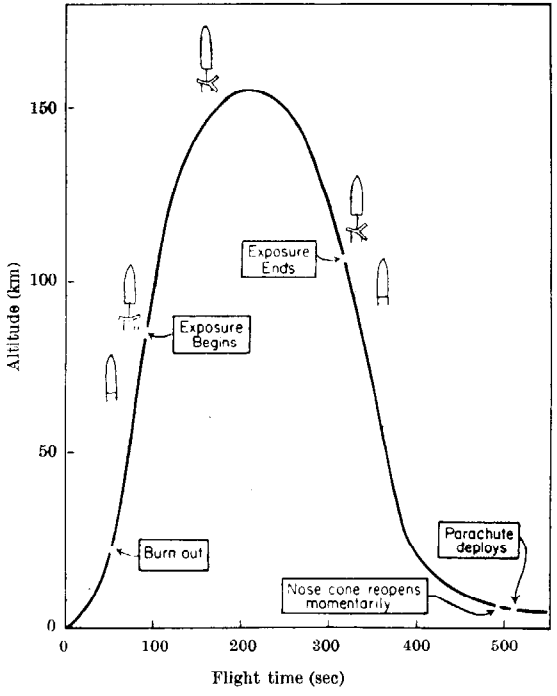

Fig. 1. Rocket altitude versus time

Table 1. VIRUS TITRES IN ROCKET EXPERIMENT

Virus	No. P.F.U. seeded	Ground set Shielded	Ground set Exposed	Flight set Shielded	Flight set Exposed
T_1-bacteriophage *	9×10^6	$8{\cdot}3 \times 10^6$	$2{\cdot}9 \times 10^6$	$2{\cdot}6 \times 10^6$	1×10^2
Polio type III †	$1{\cdot}5 \times 10^7$	$5{\cdot}3 \times 10^5$	$3{\cdot}1 \times 10^5$	$3{\cdot}3 \times 10^4$	0

* Titrated at the end of the experiment after recovery, 15 days after launch and 26 days after preparation of the dried disks.

† Titrated 22 days after launch and 33 days after preparation of the dried disks.

that re-entry heat damage to the biological samples was not serious.

On December 1, 1964, the unflown box and the flight box were returned to Albany.

Assay techniques. Fifteen days after launch, the T_1-phage samples were immersed in 10 ml. beef infusion broth for ½ h; the broth was then assayed for viable phage using the agar layer technique of Barlow[17] for T_1-phage plaque assay. Twenty-two days after launch, the poliovirus samples were washed in 10 ml. Eagle's medium containing 3 per cent foetal bovine serum and tested using the plaque assay as described by Deibel *et al.*[18,19] for poliovirus. The viability is expressed throughout as the number of infective virus particles (plaque-forming units, P.F.U.) per sample area; the number of viable virus particles originally seeded on the sample area was calculated from a similar assay of the inoculum.

Results. The microbiological results of the rocket flight are shown in Table 1. It is evident that 26 days after inoculation the ground set phage viable count showed an insignificant loss due to drying which in this experiment was remarkably low; in the case of the ground set poliovirus, the loss was much greater since the dried viable count fell by a factor of 10^4. There was no significant difference between shielded and exposed portions of either virus. The shielded sample of T_1-phage of the flight unit showed a viability not significantly different from the controls, whereas the unshielded viable count had fallen by a factor of 10^1. The unshielded count is based on a total of 10 plaques found after plating 3·4 ml. of eluate on to 18 separate Petri dishes.

The viable poliovirus fell by a factor of 10^2 in the shielded flight sample, and by a factor of at least 10^3 in the exposed sample, to a level below the threshold of the assay system which was 16 infective virus particles per cm² of sample surface.

Balloon-borne Exposure Experiment

The balloon exposure experiment was carried out as part of a Dudley Observatory micrometeorite collection experiment sponsored by the National Aeronautics and Space Administration. On December 14, 1964, the balloon was launched at the National Center for Atmospheric Research, Scientific Balloon Flight Station, at Palestine, Texas.

Sample preparation. The micro-organisms used consisted of bacteriophage T_1 and a heat-resistant *Penicillium* mould *Penicillium roqueforti* Thom. Aqueous suspensions of these organisms were placed on 450 mμ nylon-reinforced 'Millipore' filter sheets previously cemented to aluminium plates used as sample holders, and dried at room temperature. Each 'Millipore' filter sheet had an area of about 1·5 cm². Three identical samples were prepared on December 4, 1964, ten days before the flight exposure. One of the two flight samples was shielded by a sheet of approximately 2-mm-thick aluminium. The third sample served as the laboratory control.

Balloon flight exposure. The micrometeorite collector in which the biological materials were exposed was rectangular in shape and presented exposure areas of approximately 235 cm² in both the box and its cover. It was mounted and flown on top of a $3{\cdot}8 \times 10^5$ m³ balloon, while telemetry, ballast and auxiliary instrumentation were located beneath the balloon.

During launch and ascent, the collection box was locked shut. At altitude it was unlocked and opened by radio command from the ground. An independent telemetry channel indicated whether the box was fully opened or fully closed. During the flight exposure, the exposure areas were in a horizontal plane and faced upward. After the flight exposure, the box was closed and relocked by ground command. The flight was terminated by cutting off the bottom payload, and the top payload was then released and brought back to earth. Further details of this micrometeorite collection technique in which the biological exposure was carried out will be published at a later date.

The balloon and its payloads were launched at the Balloon Flight Center of the National Center for Atmospheric Research at Palestine, Texas, on December 14, 1964, at 2.40 a.m. Central Standard Time. They reached an altitude of 34·8 km at 5 a.m. C.S.T., and the exposure began at 5.05 a.m. C.S.T. over Coushatta, Louisiana. The altitude of the balloon varied from approximately 33·5 to 35 km during the exposure. During ascent the sample temperature fell to −75° C, and rose to −45° C at float altitude before sunrise. The balloon drifted rapidly eastward and sunrise occurred at about 6.15 a.m. C.S.T. After sunrise the substrate temperatures rose steadily to about 24° C and remained near that value for the rest of the flight. The samples were exposed to sunlight for about

Table 2. TITRES * IN BALLOON EXPERIMENT

Organism	No. of P.F.U. seeded	Laboratory set	Flight set *Shielded*	Flight set *Exposed*
T_1-Bacteriophage	$1{\cdot}2 \times 10^9$	5×10^4	$2{\cdot}7 \times 10^4$	$1{\cdot}2 \times 10^3$
Penicillium roqueforti Thom	$1{\cdot}9 \times 10^8$	$3{\cdot}8 \times 10^6$	$1{\cdot}5 \times 10^6$	7×10^3

* Titrated at end of experiment after recovery, 4 days after launch, 14 days after seeding.

4 h. At 11 a.m. C.S.T., the box was closed and locked. The top payload landed near Linden, North Carolina, at 12.17 p.m. C.S.T.

Results. The microbiological results of the balloon flight are shown in Table 2. In this experiment, using a different drying technique, the phage loss of the control was unfortunately much greater than in the previous one. The 6-h exposure at 34 km reduced the titre of the exposed flight set by a factor of only 10 compared with the shielded flight set. However, in the case of the *Penicillium* mould, drying caused no change in the viable count whereas the flight exposure reduced it by 10^2. With both organisms, viability of the flown shielded samples was not significantly different from the laboratory controls.

Laboratory Experiments

Experiments were performed in the laboratory in an attempt to throw light on the nature of the primary cause of the inactivation. Dry T_1-phase on 'Millipore' filter disks cemented to 'Lucite' plates was exposed to air at a temperature of approximately 150° C, as measured with a Weston '221*D*' thermometer. The sample temperature was also measured using a calibrated thermister. The result is shown in Fig. 2. After 100 sec the number of P.F.U. had dropped by a factor of 10^3 and the 'Millipore' filter started to show a slight brown discoloration. The temperature of the sample rose asymptotically and had reached 153° C at this time. No. P.F.U. were recovered after 260 sec, when the 'Millipore' filter was brown and the 'Lucite' plates were distorted considerably. By this time the temperature of the sample had risen to 180° C. Poliovirus did not survive heating under the same conditions for 20 sec.

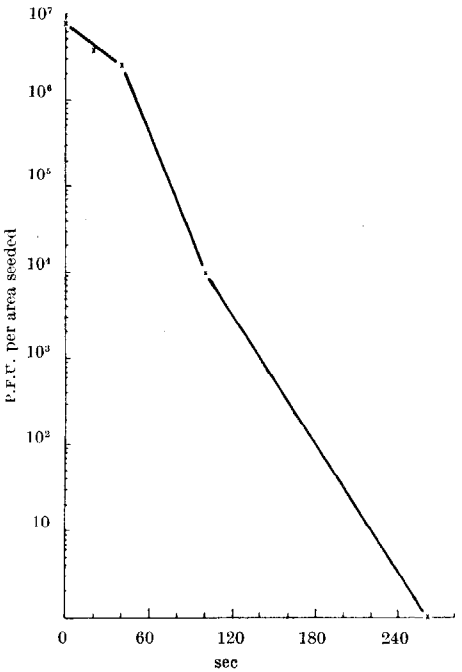

Fig. 2. Heat inactivation of dried T_1-phage lysate. Dry T_1-phage lysate on 'Millipore' filters cemented to 'Lucite' plates was exposed to air at a temperature of approximately 149° C. After 20, 40, 100, and 260 sec exposure, samples were eluted and assayed for infectivity as described in the text

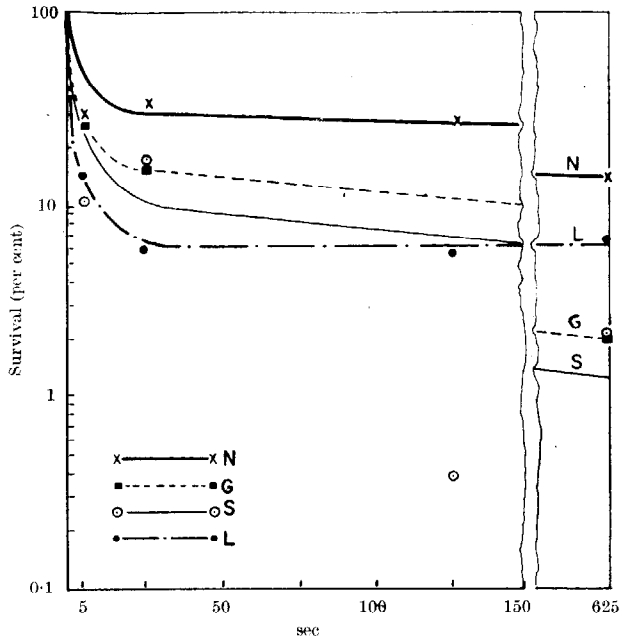

Fig. 3. Dried T_1-phage lysate after ultra-violet irradiation. Clarified (450 r.p.m. for 10 min.) T_1-phage lysate was air dried on 'Millipore' filter of 10-mμ pore size (*S*), on 'Millipore' filter of 450-mμ pore size (*L*), on glass slides (*G*), and a T_1-phage suspension containing 5 per cent yeast RNA on glass slides (*N*). From a distance of 10 cm, the samples were ultra-violet irradiated using a Westinghouse '*WL*-782-20' U-bent germicidal lamp. After 5, 25, 125 and 625 sec of exposure, samples were eluted and assayed for infectivity as described in the text

Ultra-violet light irradiation. Fig. 3 shows the ultra-violet inactivation of dried T_1-phage lysates as a function of exposure time with and without 5 per cent yeast RNA on glass slides, also of dried T_1-phage lysates on 'Millipore' filter with 450-mμ and 10-mμ porosity. The percentage of surviving P.F.U. is demonstrated. A Westinghouse '*WL*-782-20' U-bent germicidal lamp was used. The samples were kept at a distance of 10 cm from the ultra-

violet source. Fig. 4 shows the ultra-violet inactivation of purified wet T_1-phage suspensions in Eagle's medium without indicator, one of which contained 5 per cent yeast RNA. It will be noted that in all cases the ultra-violet inactivation proceeded logarithmically at first, but, after dropping by a factor of 10^1–10^2, levelled off to an almost constant value. The suspensions containing RNA showed the least inactivation.

Soft X-ray inactivation. Clarified T_1-phage lysate on 10 mμ millipore filter disks of a diameter of approximately 3 mm was air dried and exposed for 5 min to soft X-rays in a vacuum, using a Philips contact microradiograph. The potential across the X-ray tube was 1·25 kV and the tube current was 2 m.amp. Thus the organisms were exposed to X-rays of wave-lengths of 10 Å and longer. Ten identically prepared samples were used as controls and for the irradiation. After 5 min irradiation the value of the infectivity was only reduced by about 50 per cent; the average P.F.U. count per sample area fell from $2{\cdot}9 \times 10^3$ to $1{\cdot}6 \times 10^3$.

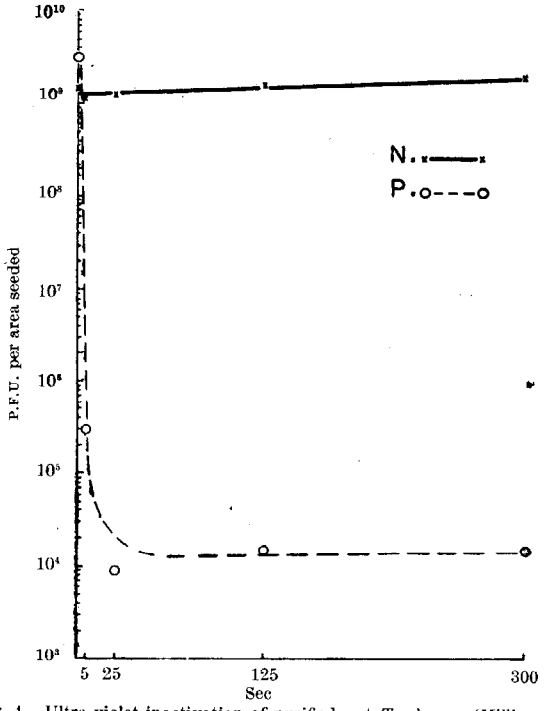

Fig. 4. Ultra-violet inactivation of purified wet T_1-phage. 'Millipore' filter disks were seeded with a purified T_1-phage suspension (*P*) and a purified T_1-phage suspension containing 5 per cent yeast RNA (*N*) in Eagle's medium without indicator. The wet samples were irradiated with ultra-violet light as described in Fig. 3. After 5, 25, 125 and 300 sec, samples were eluted and assayed for infectivity as described in the text

Discussion

While these experiments are preliminary, the complete lack of comparable data on micro-organisms fully exposed to the space environment leads us to present a brief discussion which may be helpful to other workers in this field. The main environmental factors which should be evaluated as possible causes of the loss of viability of the exposed flight samples are high vacuum, cosmic rays, ultra-violet-radiation, X-radiation and temperature extremes. In the case of T_1-phage and of *Penicillium* mould the results of the shielded samples indicate that vacuum, low temperature and cosmic rays do not represent significant lethal factors. The poliovirus, however, shows definite loss of viability in the shielded sample, possibly due to high temperature. The 'Millipore' substrate of the unshielded polio sample showed evidence of charring at the edges. The other shielded and unshielded 'Millipore' surfaces did not show any signs of heating. Under laboratory conditions, results showed that it was not possible to inactivate T_1-phage by hot air without marked discoloration of the 'Millipore' filter surface. It should be noted that this phage is reported to withstand 145° C in the dry state[20]. It seems unlikely, though not impossible, that the inactivation of the phage was due to heat; furthermore, the 38μ aluminium shield completely protected the T_1-phage sample. This shielding is believed to be too thin to give significant protection against heat, but certainly is sufficient to exclude ultra-violet light and soft X-rays which were presumably the main cause of the 10^4-fold inactivation found.

The two flight experiments were made under quite different conditions. The balloon exposure lasted nearly 6 h as compared with the 4-min duration of the rocket exposure. Furthermore, the balloon flight altitude was only 34 km whereas the rocket exposure ranged from 80 to 155 km. It is known that the intensities of ultra-violet light and of soft X-ray are considerably diminished at balloon altitudes through absorption by the high atmosphere. The greater survival of the samples during the long-term exposure on the balloon seems to reflect the greatly reduced flux of ultra-violet light and soft X-ray at balloon flight altitudes. The results of the laboratory experiments indicate that T_1-phage was not screened by the 'Millipore' filter membrane from ultra-violet light of wave-lengths close to 2537 Å (as mainly emitted by germicidal lamps) even though the average pore size was much greater (10 times) than the phage head diameter. Thus it is difficult to account for the observed inactivation of T_1-phage in the exposed rocket sample, on the basis of medium and long wave-length ultra-violet light. Furthermore, the laboratory results indicate that the germicidal ultra-violet light only reduced the titre of dried T_1-phage by a factor of approximately 10^2 and that the addition of

5 per cent RNA appeared to decrease the factor to about 10^1; whereas purified wet T_1-phage is completely protected by 5 per cent RNA. This accords with the concept that crude lysate has considerable shielding ability against ultra-violet light of this wave-length. It also seems to indicate, as suggested here, that the 10^4-fold inactivation of broth lysate T_1-phage exposed in the rocket experiment was only partially due to ultra-violet light of ordinary germicidal wave-length. It appears that the observed inactivation of T_1-phage was due to electromagnetic radiation from the Sun, of wave-lengths shorter than 2600 Å, but not sufficiently energetic to penetrate 38μ of aluminium foil.

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